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CONTROLLING CABBAGE APHID (*Brevicoryne brassicae* L.) USING ISOLATED MYCOINSECTICIDES

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ABSTRACT

This study aims to isolate and identify entomopathogenic fungi and evaluate their virulence against the cabbage aphid, *Brevicoryne brassicae* L. A weekly survey of pests present on cabbage crop at El Dair region, Qualubia Governorate was carried out during 2014 – 2015 season. The results indicated that, cabbage aphid, *B. brassicae*, was moderate collected. The susceptibility of cabbage aphid *B. brassicae*, for two entomopathogenic fungi, *i. e.*, *Beauveria bassiana* and *Metarhizium anisopliae*, were investigated. Where, *B. bassiana* gave higher levels of mortality against adults of cabbage aphid with low lethal time (LT_{50}). The mortality percents of both entomopathogenic fungi were increased with increasing the period after treatment.
Keywords: Cabbage crop, entomopathogenic fungi, *Brevicoryne brassicae* (L.).

INTRODUCTION

Cabbage aphid, *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) is an important agricultural insect pest that attack many vegetable crops all over the world (Irshad, 2001). In some cases, the vegetable crop can be completely loss due to heavy infestation, while the aphid can reduce the crop yields due to sucking the plant sap besides excretion on honey dew which inhibit photosynthesis process leading to retardation in the plant growth. Also, aphid is well known as a main plant pathogen transmitter (Strauss *et al*., 2002 and Asi *et al*., 2009).

The indiscriminate use of insecticides for controlling these devasting pests caused several environmental problems such as pollution besides the negative effects on the other beneficial insects (Asi *et al*., 2009). These problems derived the scientists for finding more safe methods in controlling these pests. Entomopathogenic fungi, especially *B. bassiana* and *M. anisopliae* seems to be more safe and good alternatives in controlling these pests (Loc *et al*.,2010).The results of many researches assured successful safely use of the previous species of entomopathogenic fungi in controlling several insect pests rather than the pest under studying (Khashaveh *et al*., 2008 Barra *et al*., 2013). In this research infested aphids with *B. bassiana* and *M. anisopliae* were collected from the field. The two species of entomopathogenic fungi were propagated in the laboratory. Mortality effects and virulence of both species were evaluated against cabbage aphid.

MATERIALS AND METHODS

Survey:

Cabbage plants growing at El-Dair region, Qualubia Governorate, Egypt were surveyed during 2014 – 2015 season. Three plots (A, B & C) were sampled weekly and all cabbage aphid, *B. brassicae* adults were collected from 724 randomly selected plants (Embaby and Lotfy, 2015). Several aphid colonies were surveyed according to (Strauss *et al*., 2002) method. The collected aphids were placed individually as adult into empty plastic tubes with a small slice of leaf from the host plant. All collections were held in the laboratory at National Research Centre, Plant Protection Dept., Egypt, to record the identification of the insects. Collections were checked every 2-3 days to record the number of dead adults and to add fresh food material if required. The dead insects were collected.

Isolation and identification of fungi: The fungi were isolated and identified to culture according to methods of Boikova and Novikova, (2001). The cultures of fungi were grown in sterilized Petri dishes on agarized media at 24± 2°C using Sabouraud's nutrient media then identified according to the manual of Polovinkoa *et al*. (2010).

Fungus Culture: Two isolates of entomopathogenic fungi namely *Beauveria bassiana* and *Metarhizium anisopliae* were cultured on potato dextrose agar medium containing 20g glucose, 20g starch, 20g agar, and 1000 ml of distilled water in test tubes. Then autoclaved at 121^0C (15 Psi) for 15-20 minutes and incubated for two weeks at 26±1ºC.Conidia were harvested by brush, used as stock and stored in refregirator at 4ºC, from which the fungi were used as inoculum for laboratory experiments. Bioassay procedure for efficacy of entomopathogenic fungi against *B. brassicae* was followed.

Pathogenicity: The 16-days-old cultures of *M.anisopliae* and *B.bassiana* were used for the test. Adult stage of the cabbage aphid, *B. brassicae* was bioassayed. The majority of the test-insects fed on the leaves of their host plants. The tested insects were infected using the contact method by placing 45 adult aphids in Petri dishes directly on the surface of conidial layer of each fungus isolate alone for 60 seconds. The development of mycosis in the tested-insects was observed at 20–25°C. The virulence of the isolates were estimated by two parameters: death rate of the infected insects (%) and time of their death (days) according to (Polovinkoa *et al*., 2010)

Data analysis: The lethal time (LT_{50}), the number of days until death of 50% the adult was computed through probit analysis using the Propan Propgram according to (Finney, 1970).

RESULTS AND DISCUSSION

1-Survey:

Survey of cabbage aphid on cabbage plants were recorded in Table (1).Seven hundred and twenty four

plants were surveyed and incidence of infested cabbage plants by cabbage aphid was 22.422%.On the other hand location, (B) showed higher infection than others which recorded 10.20%, followed by (A) which gave 7.604%, while location (C) was with less incidence and recorded 4.62%.

Table (1): Percent infestation of cabbage plants by cabbage aphid in different sampled locations

Percentage of Infestation	Total No of		Total No of examined plants	Location
	Healthy plants	Infested plants		
7.60%	243	20	263	A
10.20%	220	25	245	B
4.62%	206	10	216	C
22.42%	669	55	724	Total

2-Fungal infestation:

Naturally occurring entomopathogenic fungi which were isolated from adult cabbage aphid tabulated in Table (2). *Metarhizium anisopliae*, was the isolated entomopathogenic fungus, whereas the representatives of the genera *Aspergillus*, *Mucor*, and *Rhizopus* can be conditionally attributed to saprotrophic fungi that more often develop on the died insects.On the other hand, *M. anisopliae* was the most occurring fungus comparing with other isolated fungi recording 92.25% followed by *Rhizopus* sp. (5.53%) and *Mucor* sp. (1.85%), and *Aspergillus niger* (0.37%). Polovinkoa *et al*. (2010) reported that, naturally occurring entomopathogenic fungi have been shown to occasionally cause high mortality of lepidopterous larvae in cabbage crop. The mycobiota of the collected cadavers of insects lists ascomycetes anamorphs of 13 genera. Such species as *B.bassiana* and *M.anisopliae*, are entomopathogenic fungi, whereas the representatives of the genera *Aspergillus*, *Fusarium*, and *Rhizopus* can be conditionally attributed to saprotrophic fungi that more often develop on the insects who died due to other reasons. Although in some cases the species and strains *Aspergillus* and *Fusarium* are experimentally shown to be highly pathogenic for insect, it is known that the given fungi usually develops on the insect that died due to mechanic damage to cuticle.

Table (2): Percentage of fungal frequency associated with adult aphids.

Type of fungi	Total of fungi	% of fungal frequency
Aspergillus niger	2	0.37
Metarhizium anisopliae	500	92.25
Mucor sp.	10	1.85
Rhizopus sp.	30	5.53
Total	542	100.00

3- Pathogenicity to cabbage aphid:

percentages of cabbage aphid, *B. bassiana* adult mortality were recorded in Table (3) and represented in Figs. (1, 2 and 3). Data indicated that, *B. bassiana* gave higher levels of mortality while, low level of mortality was recorded with *M. anisopliae* as follow:-

a- After Four days from treatment, *B. bassiana* and *M. anisopliae* resulted in 88.89%, and 51.11% mortality of cabbage aphid adults, respectively.

b- After Seven days from treatment, both fungal isolates caused mortalities. But *B. bassiana* was more effective (100% mortality) than *M. anisopliae* (80% mortality) against adult aphids.

c- After ten days from treatment, all fungal isolates again caused mortalities after 10 days of treatments. *B. bassiana* isolate, was highly effective against aphid with 100% mortality than *M. anisopliae* (91.11% mortality)

On the other hand, the same Table (3) shows that, the levels of mortality percent were increased with increasing the period after treatment. Mortality percent of cabbage aphid was found to be increased from 88.89% after 4 days to 100% after 7days when treated by *B. bassiana* fungus. Percentage of cabbage aphid mortality was found to be increased from 51.11% after 4days to 80.00 and 91.11% after 7 days and 10 days respectively, when treated by *M. anisopliae*. This result about agreed with (Kumar and Chowdhry, 2004) who reported that *M.anisopliae* gave mortality percentage ranged between 50-92.5%. Thompson and Brandenburg (2005) reported that death caused by the fungi usually was more than 48 post infection after attachment of conidia to the insect cuticle.

Table (3): Cummulative corrected mortality percentage of cabbage aphid, (*B. brassicae*) using some mycoinsecticide (as a biopesticide)

Fungal isolates	Total Number of treated adult aphids	Period after treatment (days)					
		4		7		10	
		Total No. of dead adults	% mortality	Total No. of dead adults	% mortality	Total No. of dead adults	% mortality
B. bassiana	45	40	88.89	45	100	45	100
M. anisopliae	45	23	51.11	36	80.00	41	91.11
Total	90	63	70.00	81	90.00	86	95.56

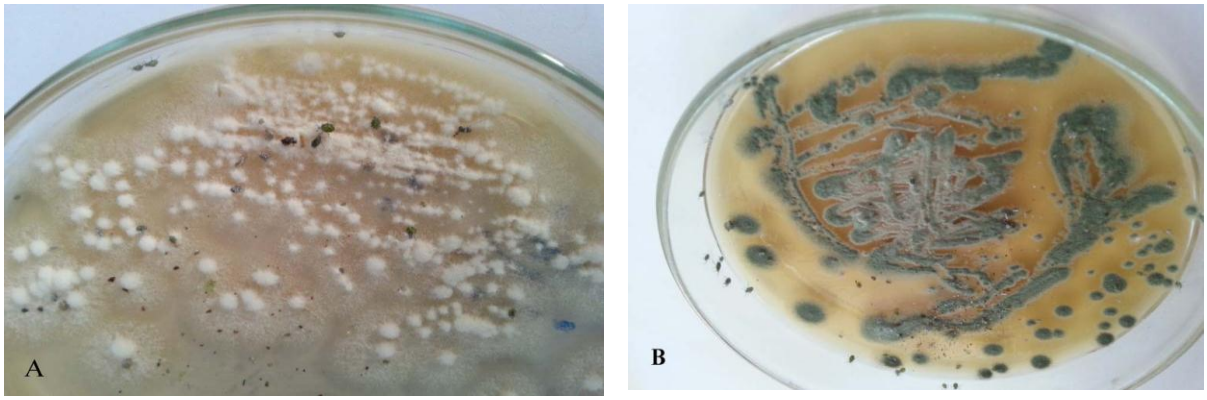

Fig.1: Pathogenicity: Cabbage aphid, adult instars were infected using the contact method on the surface of conidial layer of both the entomopathogenic fungi *B. bassiana* (a) and *M. anisopliae* (b)

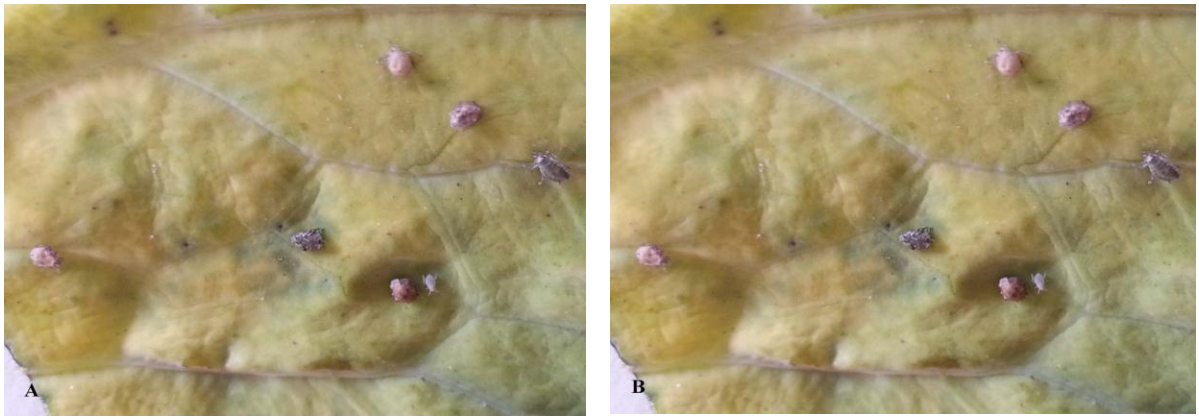

Fig.2: Cabbage aphids on cabbage leaf, insect mortality and the mummies (a) caused by *B. bassiana* fungus, insect body after death, the fungus forms aerial mycelia and sporulation. The insects which emerged from the mummies were killed (b).

Fig.3: Adult aphids dead by *M. anisopliae* fungus

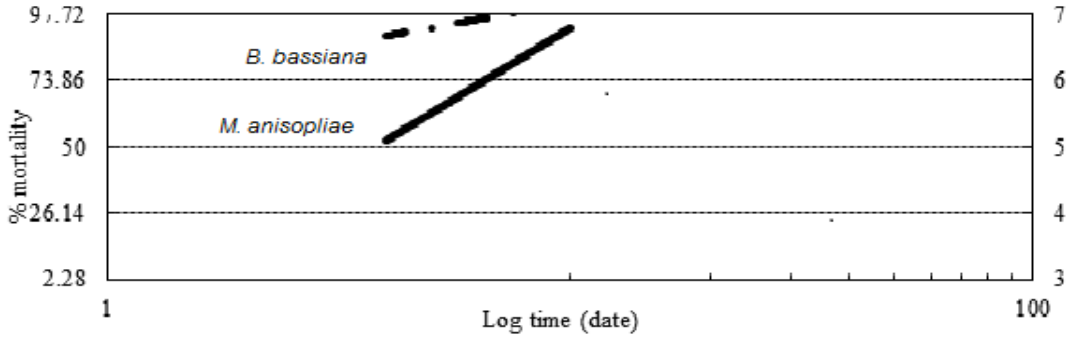

Fig (4): log probit toxicity lines of of *M .anisopliae* and *B. bassiana* against the adult aphid expressed as LT_{50} values. Where, lethal time required to kill 50% of the aphid for *M. anisopliae* was 3.92 days and 3.53 days for *B. bassiana* as Fig (4).

Ramanujam *et al*. (2014) stated that, entomopathogenic fungi from hypomycetes group are opportunistic pathogens and usually cause insect mortality by nutritional deficiency, destruction of tissues and by release of toxins. Cuticular degrading enzymes of entomofungal pathogens like chitinase, protease and lipase play an important role in the pathogenicity of these organisms on insects in the breakdown of insect cuticle for penetration of fungal germ tube into the insect body. The entry of entomopathogenic fungi through the insect cuticle is considered to occur by a combination of mechanical pressure and enzymatic degradation. Several mycotoxins like, Beauvericin, Beauverolides Bassianolide (by *B. bassiana*, *V. lecanii*, *Paecilomyces* spp.) and Destruxins A, B, C, D, E, F (by *M. anisopliae*) are produced during pathogenesis and these act like poisons for the insects. After the death of the insects, the fungus breaks open the integument and forms aerial mycelia and sporulation on the cadavers. The fungi of entomopthorales group are obligate pathogens of insects and cause host death by tissue colonisation with little or no use of toxins. Hajek, and Leger (1994) reported that, entomopathogenic fungi involve an infective spore stage, in which it germinates on the host cuticle, forming a germ tube that penetrates the cuticle and invades the haemocoel of the insect host. (Goettel *et al*., 2010); (Rohlfs and Churchill, 2011) and (Safavi, 2013) stated that, the fungus then multiplies within the insect body and kills it. Death occurs due to toxin production by the fungus and/or multiplication to inhabit the entire insect body. Entomopathogenic fungi are prolific producers of bioactive secondary metabolites, which are predicted to play key roles as virulence factors for fungi, infecting arthropods. Metabolites produced by entomopathogenic fungi would serve one or more of the following functions: (1) toxic to the host and help to cause death; (2) to aid the fungus overcome host defence; (3) to suppress competition from other pathogens and saprophytes on the insect cadaver; (4) to provide a defence outside the host against mycophagous organisms. Accordingly, many secondary metabolites tend to be compounds that bear toxic or inhibitory effects on other organisms.

Also, (Zimmermann, 2007 and Safavi, 2013) reported that, entomopathogenic fungi produce secondary metabolites which may bioactively help fungus in its virulence toward insect hosts. amajority of these insecticidal molecules are low molecular weight secondary metabolites. Beauvericin, bassianin, bassianolide, beauverolides, beauveriolides, tenellin, oosporein, oxalic acid bassiacridin are some of the important metabolites of *B. bassiana*. Among them, Beauvericin is the most important compound which was reported first from *B. bassiana*. Beauvericin is a toxic cyclic hexadepsipeptide and comprising a cyclic repeating sequence of three molecules of N-methyl phenylalanine that alternate with three molecules of 2-hydroxyisovaleric acid. Not all isolates of *B. bassiana* produce beauvericin in vitro. Nevertheless, there are some reports of no toxicity against certain insects.

CONCLUSION

The obtained results in this study explore the pathogenicity of the entomopathogenic fungi *M.anisopliae* and *B.bassiana* as safe and good insecticides alternatives for controlling against Cabbage aphid (*Brevicoryne brassicae* L.)

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مكافحة من الكرنب باستخدام بعض المبيدات الحيوية

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في هذا البحث تم دراسة تاثير بعض سلالات الفطريات الممرضة للحشرات والمعزولة من حشرة من الكرنب المصابة بالفطر علي نسبة الموت للمن بعد معاملتها بهذة السلالات . اوضحت النتائج ان فطر بيوفاريا باسيانا كان اكثر فاعلية من فطر الميتارزيم انسيوبليا حيث اعطي نسبة موت وصلت الي ١٠٠% لحشرة المن المعاملة. اوضحت الدراسة ايضا ان فطر البيوفاريا كان اكثر فاعلية حتي بعد ١٠ ايام من المعاملة حيث ظلت نسبة الموت في حشرات المن المعامل كما هي ١٠٠% بينما قلت هذة النسبة مع فطر الميتارزيم حيث وصلت نسبة الموت بعد ١٠ ايام من المعاملة ١١.٩١%.